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Cardiac Teratogenesis of Trichloroethylene and Dichloroethylene in a Mammalian Model

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Recent epidemiologic studies have demonstrated a greater than expected number of pediatric patients with congenital heart disease in areas where drinking water was contaminated by halogenated aliphatic hydrocarbons. Trichloroethylene, trichloroethane and dichloroethylene were the principal contaminants in the groundwater. A previous study of chick embryos demonstrated that when injected into the air sacs of fertilized eggs trichloroethylene produced more than three times the number of cardiac defects that are found in control embryos.

This mammalian study demonstrates similar effects of trichloroethylene and dichloroethylene when applied under provocative circumstances (that is, solutions delivered through a catheter into the gravid uterus from an intraperitoneal osmotic pump) to the developing rat fetus in utero during the period of organ differentiation and development. Furthermore, the effect is dose dependent for both agents. Although only a very small number of congenital heart anomalies (3%) were found in the control group, 9% and 12.5% were found in the lower dose trichloroethylene and dichloroethylene groups and 14% and 21% in the higher dose groups, respectively ($p < 0.05$).

A variety of cardiac defects were found. Dichloroethylene appears to be at least as great a cardiac teratogen as trichloroethylene even though it was administered at a 10-fold lower concentration. These agents appear to be specific cardiac teratogens because only a single noncardiac anomaly was found.

This study in a rat model demonstrates a dose-dependent relation between fetal exposure to trichloroethylene and dichloroethylene in utero during the period of organogenesis and the appearance of a variety of congenital cardiac defects.

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Trichloroethylene and closely related compounds are among the most common water supply contaminants in the United States and abroad (1). Although considerable information is available regarding the short- and long-term toxicity of these agents and to a lesser extent their general teratogenicity, little is known about their specific cardiac teratogenesis.

Two previous epidemiologic studies (2,3) found an association between these halogenated hydrocarbons and an increased incidence of major cardiac malformations in children born to mothers who lived in areas of water contamination. In one of these studies (2), the California Department of Health Services investigated potential problems induced by entry of trichloroethane from a holding tank into the water supply in an area of Santa Clara County. Analysis of the water indicated the presence of both trichloroethane and dichloroethylene. A greater than expected number of major cardiac congenital anomalies was found in children born to mothers who lived in the area. In the other study (3), trichloroethylene was known to have entered the groundwater in an area of Tucson, Arizona during the 1950s, contaminating numerous wells. Water contamination (by trichloroethylene, dichloroethylene and chromium) was detected in 1981 and affected wells were later closed to human consumption. University of Arizona pediatric cardiologists had noticed since 1973 that many of their patients with congenital heart defects lived in the area served by the contaminated wells. An epidemiologic study (3) revealed that the relative incidence of congenital heart disease during the period of water contamination in this study (1969 to 1981) was three times as high in the contaminated water area as in other areas. These case-control epidemiologic studies, although suggestive of an association of the halogenated contaminants

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and congenital heart disease, were not designed to establish a cause and effect relation.

Subsequently, a study (4) was undertaken to determine the teratogenicity of trichloroethylene in early embryogenesis in an avian model. Data from this study established this agent as an avian teratogen, with significantly increased numbers of malformations, resorptions of embryos and embryonic deaths in treated groups compared with control groups. The specific cardiac teratogenicity of trichloroethylene in the avian model was examined in the same study; again, embryos treated with this agent developed more than three times as many congenital heart deformities as were developed by control groups, and trichloroethylene was established as a cardiac teratogen in an avian model.

The present study was designed to determine whether trichloroethylene or dichloroethylene causes cardiac teratogenicity in the mammalian fetus under the most provocative of circumstances (that is, direct intrauterine exposure of fetuses during the critical days of cardiac organogenesis). A rat model was selected because of the very low incidence of spontaneous cardiovascular anomalies (5).

Methods

Study group. This study conformed to the Position of the American Heart Association on Research Animal Use, adopted November 11, 1984. The study group consisted of 70 young sexually mature Sprague-Dawley female rats weighing 250 ± 30 g and 4 fertile Sprague-Dawley male rats. All were fed Teklad 4% Mouse/Rat Diet ad libitum. When in an appropriate stage of estrus (determined by daily vaginal smears), a female rat was placed overnight in a separate breeding cage with a male rat. Pregnancy was established by detection of spermatozoa on vaginal smears.

Administration of agents. Trichloroethylene and dichloroethylene (1,1) were obtained from Aldrich Chemical Co. and diluted to the required concentrations in 0.9% sodium chloride solution for intrauterine administration. Female rats were then divided into five groups for administration of control or test agents including physiologic (0.9%) saline solution control (Group 0), 1,500 ppm trichloroethylene in saline solution (Group 1), 15 ppm trichloroethylene in saline solution (Group 2), 150 ppm dichloroethylene in saline solution (Group 3) and 1.5 ppm dichloroethylene in saline solution (Group 4). Solutions were delivered by an Alzet osmotic minipump (model 2002) filled to 200 μl with the appropriate solution for each group. Delivery rate was 0.5 μl/h from each pump over a 2 week period. Distribution and delivery of pump contents were confirmed by substituting methylene blue solution in one instance and demonstrating staining of all fetal membranes, placentas, uterine horns and vaginal vault.

Experimental preparation. On day 7 of pregnancy, the female rat was anesthetized with ether. With use of a sterile technique, a ventral midline incision was made on the linea alba and the uterine horns were located. Once a gravid uterus was observed, a pursestring suture of 5.0 chromic gut was placed in the serosal layer of the distal uterine horn. An 18 gauge needle was used to incise the uterus and open the center of the preplaced purse string. A 3 cm Silastic 0.030 catheter leading from a filled Alzet minipump was passed into the uterine lumen, and the pursestring suture was then secured to fix the catheter in the uterine horn. The same procedure was repeated for the opposite horn. Once both pumps were in place, the abdominal wall was closed with simple interrupted 5.0 gut sutures, and the skin was closed with 9 mm autoclips. Recovery from anesthesia was speedy, and rats were ambulatory after 30 to 90 s. Weight was recorded daily and health during pregnancy was closely monitored.

Examination of fetuses. On day 22, approximately 1 day before parturition, the pregnant rat was killed in a carbon dioxide chamber. The gravid uterus and ovaries were removed through a ventral midline incision and examined. Each horn was opened along its length, allowing visualization of all fetuses and implantation and resorption sites. Placental weight, crown-rump length and weight of each fetus were recorded. Each fetus was examined for external abnormalities. A "V" type incision was made in each fetus, starting at the distal sternum and extending to each axilla to expose the thoracic cavity. The great arteries and veins were observed in situ. The nature of the pulmonary venous attachment to the left atrium and the inferior and superior venae cavae connections to the right atrium were determined under visual magnification before removal of the heart. The heart was then flushed with 2% gluteraldehyde solution by means of an apically placed 27 gauge needle, fixed in 2% gluteraldehyde for 24 h and transferred to a 0.1 mol/liter phosphate buffer for storage. Each heart was identified only by a seven digit code number.

Evaluation of individual hearts included gross morphologic and representative histologic study. The 3 to 4 mm heart was dissected with a Nikon SMZ-2T light microscope with a TV monitor that allowed excellent visualization and manipulation. Initially, the heart was examined for any gross abnormalities as viewed from both dorsal and ventral aspects. The right atrial appendage was then excised to evaluate the atrial septum for defects. If adequate visualization of the atrial septum was not produced, the left atrial appendage was also removed. The aorta and pulmonary vessels were evaluated for course, caliber and orientation and then excised at their valve rings. All remaining atrial tissue was removed and the pulmonary, aortic, tricuspid and mitral valves could then be clearly seen. The location of the coronary ostia was noted. Each valve was probed for patency, and the formation of each valve leaflet was carefully observed.

The heart was then placed in a series of dehydrating

Table 1. Cardiac Anomalies in 295 Fetuses Exposed In Utero to Trichloroethylene (TCE) and Dichloroethylene (DCE)

	Saline Solution	DCE (ppm)		TCE (ppm)	
		1.5	150	15	1,500
Gross arterial			1	1	
Gross cardiac morphology		1			
Atrial septal defect		3	4	1	4
Mitral valve		1	1	1	1
Tricuspid valve		1		1	1
Atrioventricular canal		2	2		1
Ventricular septal defect					
Membranous	1	3	4		
Muscular			1		
Single ventricle					1
Malposed truncus arteriosus					1
Pulmonary valve			4	2	3
Aortic valve	1		2		4
Total no. of hearts examined	65	64	65	45	56

alcohols in preparation for critical point drying in a Samdri dryer. Under light microscopy, the free left ventricular wall was removed, exposing the ventricular septum and the mitral valve. The free right ventricular wall from the tricuspid valve to the pulmonary valve was then removed. Both sides of the septum were examined. Abnormalities, determined both independently and collectively by three of the authors (a pediatric cardiologist, a pathologist and a veterinarian), were then photographed with a Nikon N2020 camera mounted on the light microscope. Each heart was examined for abnormalities, as listed in Table 1. Decoding occurred only after final evaluation of all fetuses and hearts.

Results

Pumps and delivery catheters in all maternal rats were in place at term and no maternal abnormality was associated with surgery or treatment. No significant fibrosis or adhesions occurred around pumps or catheters. Ovaries had normal morphologic features.

Study groups. There were 10 to 17 maternal rats in each group. The 62 maternal rats had a range of 0 to 16 fetuses each. There was a mean of 139 implantation sites for all groups. Litter size showed no correlation with treatment or dose. A total of 373 live fetuses were examined (mean/group = 75). Careful gross morphologic external inspection demonstrated only one noncardiac congenital anomaly, namely, agnathia in a member of a treated group.

Three types of data were compiled for both trichloroethylene and dichloroethylene: 1) in utero abnormalities observed when the uterine horns were examined on day 22 just before delivery (Fig. 1); 2) percent congenital fetal cardiac anomalies calculated on a group basis (Fig. 2); and 3) types of cardiac anomalies (Table 1).

In utero abnormalities. Figure 1 shows the percent of implantation sites (that is, sites that did not appear to develop beyond implantation and contained a metrial gland only), resorption sites (that is, sites where development began but resorption later occurred) and dead and live fetuses for each group. The consistency in all four of these categories for all groups demonstrated that 1) toxic doses of each agent were not delivered to any group; 2) at study dosages, both agents permitted fetal development in utero; and 3) treatment with either agent produced no statistically significant in utero differences among groups.

Number of fetal cardiac abnormalities. Figure 2 shows significant differences in the percent of abnormal hearts among groups. Variations from normal morphology similar to those found in humans were not classified as defects (for example, tricuspid valve leaflet contribution to complete coverage of membranous ventricular septum). A total of 295 hearts were examined (fewer than the number of live fetuses produced because one fetus from each litter was saved for quantitative evaluation of trichloroethylene or

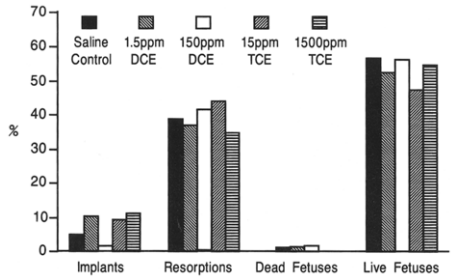

Figure 1. Percent of in utero abnormalities in rats exposed to trichloroethylene (TCE) or dichloroethylene (DCE) by means of intrauterine pumps, illustrating the lack of statistical difference between treated and untreated groups.

dichloroethylene content). Because of the rapid in vivo disappearance of both agents with time and the very slow delivery of these compounds through the minipumps, tissue levels were not detectable by gas chromatography. The mean number of hearts examined per group was 60. Only after unanimous agreement by the investigators and photography for documentation was a heart defect assigned to an abnormal group.

Only a small number of hearts (n = 11) had more than one defect or defect complex. The control group demonstrated 3% defective hearts. Low dose (15 ppm) trichloroethylene (Group 2) produced 9% defective hearts (p = 0.18) and the high dose (1,500 ppm) (Group 1) resulted in 14% defective hearts (p = 0.03). Low dose (1.5 ppm) dichloroethylene (Group 4) produced 12.5% defective hearts (p = 0.045) and the high dose (150 ppm) (Group 3) resulted in 21% abnormal hearts (p = 0.001). Analysis of variance of the combined experimental groups compared with the control group resulted in a p value <0.00005. Both agents demonstrated dose-related effects. In each case, a 100-fold increase in dose resulted in a >6% increase in the percent of defects.

Types of cardiac defects (Table 1). No particular individual abnormalities or combinations of defects were selectively induced by either agent. The most common were atrial septal defect (12 in all groups combined), pulmonary valve anomalies (9), aortic valve anomalies (6) and membranous ventricular septal defect (7).

Discussion

The most important finding of this study is the selective cardiac teratogenicity of trichloroethylene and dichloroethylene in a mammalian model. This is the first mammalian study to evaluate in a precise manner the cardiac teratogenesis of these agents. Dichloroethylene exposure produced cardiac abnormalities in the same range as trichloroethylene, but at a concentration 10 times less than that of the latter.

Previous teratogenic studies. Considerable biologic information regarding trichloroethylene is available, but less is available for its natural breakdown product dichloroethylene. Briefly, imbibed trichloroethylene is almost completely absorbed by the gut and metabolized in the liver to trichloroethylene-glucuronide, trichloroethanol, trichloroacetic acid, oxalic acid and 2-hydroxy-acetylethanolamine (6). Excretion of metabolites by the kidney is completed within 3 days (7). Metabolites have been shown to cross the placenta into fetal circulation and amniotic fluid (8–11). Trichloroethylene exposure has been implicated in abnormal spermatogenesis (12), and the compound has been shown to concentrate in the ovary (13). Studies of noncardiac teratogenesis in mammals have produced inconsistent findings. Four trichloroethylene inhalation studies (14–17) failed to show teratogenesis in rats; however, because this agent is water soluble only at relatively low concentrations (0.107 g/100 ml at 20°C, approximately 1,070 ppm) and is volatile, it is difficult to assess the extent of fetal exposure. A teratologic study (16) of this agent in rabbits demonstrated production of hydrocephalic fetuses. Using an avian model, however, other investigators (18) demonstrated that all experimental embryos and no control embryos had malformations. Reevaluation of one other such study (19) showed some similarities. Inhalation of trichloroethylene was studied but with no mention of cardiac morphology. One study (14) included gross examination of all fetal organs and microscopic examination of fetuses but did not specifically address the cardiac abnormalities. Many of our findings would not have been revealed by these methods. In one study (20), a teratogenic effect was not found in rats or rabbits inhaling up to 160 ppm dichloroethylene or ingesting 200 ppm of the agent. However there is no mention of detailed examination or dissection of the heart. Other findings are consistent with those in the present study.

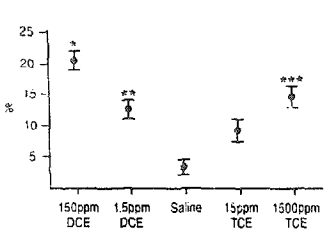

Figure 2. Congenitally defective hearts in rat fetuses exposed to trichloroethylene (TCE) or dichloroethylene (DCE) in utero, expressed as a percent of each treatment group. Standard error is designated by the limit markers. *p = 0.001; **p = 0.045; ***p = 0.03. Analysis of variance: p < 0.00005.

Investigators have studied the general teratogenicity of trichloroethylene in small mammals, but none have specifically designed their study to address effects on the developing heart. The first study (4) of specific cardiac teratogenesis of this agent was triggered by the two epidemiologic studies (2,3) in California and Arizona that suggested an association between trichloroethylene or dichloroethylene and their combination and clusters of human congenital cardiac anomalies. That study (4) used a White Leghorn chick model. Eggs were incubated and injected at various stages with concentrations of trichloroethylene ranging between 5 and 25 mmol/liter. Mineral oil and saline solution were used as control agents. This study, performed at stage 18 in an avian model, established this agent as a general avian teratogen and a cardiac teratogen.

The present study. Findings in the present study using a mammalian model can be directly compared with those of

the avian study with respect to four major categories: 1) In the chick study, increased numbers of dead, resorbed and malformed embryos were found in the trichloroethylene group as compared with the control group. In the mammalian study, no significant difference in these variables was found for trichloroethylene or dichloroethylene as compared with the saline-treated control group. In this respect, our finding is consistent with prior negative general (noncardiac) teratogenesis studies (14–17). 2) Comparison of cardiac findings in the present study correlated well with those in the chick study. Compared with control chicks, more than three times as many chicks exposed to trichloroethylene had an abnormal heart. In our rat study, a similar or greater increased ratio was found in all groups. 3) A dose-dependent relation was found in the rat model but not in the chick model. 4) No particular defect predominated in either the avian or the mammalian model. Results relating to nonfocused cardiac lesions were consistent with the epidemiologic studies. The 3% rate of cardiac abnormality found in the rat control group is a little higher than that found in subsequent experiments (1.5% or 2.5% if isolated positional abnormalities are included). The slightly higher rate of cardiac abnormality may be associated with surgical manipulation in this study. In both the chick and the rat studies, cardiac abnormalities were found only after detailed dissection.

Limitations of study. The present study has several important limitations. Although trichloroethylene and dichloroethylene were the test agents, the effect could have resulted from either the agent itself or metabolites of the agent. Second, certain cardiac and great vessel abnormalities may not have been detected. Examples of abnormalities that were not studied include coarctation of the aorta, regurgitant valves and coronary artery distribution beyond the ostia. It is important to indicate that results of this study were achieved by a very provocative test, bathing fetuses in relatively high concentrations of both agents. In the epidemiologic studies, concentrations of trichloroethylene in drinking water were approximately 260 ppb or less and dichloroethylene concentrations were about 10% of those of trichloroethylene. A search for the mechanism was not an objective of this study; however, it would be an interesting corollary. Accordingly, this provocative test establishes only that either agent individually has the capability to produce selective cardiac teratogenesis; it in no way proves that human cardiac teratogenesis is caused by drinking water contaminated by these agents.

Conclusions. The aim of this study was to determine whether trichloroethylene or dichloroethylene under most conducive conditions could produce specific cardiac teratogenesis in the rat. Our findings support this hypothesis in relation to both agents; a dose-dependent relation occurred with both.

Extrapolation of these results directly to human exposure may be inappropriate. In this provocative study, dosages were higher than those found in even the most contaminated wells to which humans were exposed. Further, exposure was directly in utero, bypassing maternal circulation and the placental barrier during the phase of organogenesis when the heart would be most vulnerable (days 8 to 14). However, these cautions do not detract from the finding that these halogenated hydrocarbons produce selective cardiac teratogenesis in a mammal.

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